NOTES ON *VIBRIO DENITRIFICANS*, SEWERIN.

By R. Greig Smith, M.Sc., Macleay Bacteriologist to the Society.

(Plate xi.)

In examining the bacteria contained in a sample of disintegrating cement which was obtained from one of the canals used for conveying the Sydney Water Supply, an organism which had some curious features was isolated. It is identical in form with *Rhizobium leguminosarum*, the nodule organism of the Leguminosæ, but differs from it in the power of growing on ordinary media as well as in media containing little nutriment. Although so like the nodule organism which is supposed to convert free nitrogen into combined forms or to assist the plant to do so, this organism does almost exactly the opposite and reduces nitrate to nitrogen gas.

The investigation of the organism in subculture showed it to be *Vibrio denitrificans*, Sewerin, but for some time it could not be identified with this organism, because a true vibrion form could not be observed. By using a low magnification and observing overstained films, bent forms can be seen, but by examining a properly stained film with the oil-immersion (No. 4 ocular and $\frac{1}{12}$ oil objective, Leitz) the bent forms are clearly seen to be double organisms bent at the point of attachment. The individual cells are straight.

Like *Rhizobium leguminosarum*, the organisms appear in a variety of forms, as the coccus (0·8 μ), oval cells (0·6 : 1·0 μ), rods with rounded ends (0·6 : 2 μ), exclamation mark (!), conical cells, γ, Y and double cells bent at an angle. They stain readily with

carbol-violet, and show unstained portions which are not constant in location, being generally between the middle and end of the cell. In the Y-shaped forms the compound nature may be discerned, and it is seen to consist of three organisms enclosed in a branching capsule or tube. The γ-forms are made up of a rod and one or two smaller cells; the latter may spring from the end of the rod perpendicular to the plane of the film, and become bent over at right angles, or nearly so, during the process of drying the coverglass. The various forms are most numerous in cultures upon solid media containing potassium phosphate. Such a medium can be prepared by adding 10% gelatine or 2% agar to the peptone-glucose solution recommended in a former paper.* In a two days' culture upon this gelatine medium all the variety of forms can been seen. The plate which accompanies this paper was prepared from a film of such a culture; the cells were stained with carbol-violet.

The organisms as observed in the hanging drop are motile, spinning round and darting about the microscopic field. The flagella are generally two in number and located at one end of the simple cell, but they also occur singly at one end, and sometimes at both ends, of the organism.

The optimum temperature is 28-30° C., and although it grows at 37° on solid media the growth is restricted. In opposition to Sewerin I find that there is practically no growth in nutrient or nitrate bouillon at 37°. Under anaerobic conditions, it forms a scanty growth on agar. On ordinary acid potato the growth is luxuriant, moist glistening, creamy-white and spreading; the colour ultimately becomes brownish-yellow. Sewerin† in his first paper said no growth occurred on potato, and in his second that there was formed a narrow, flat, yellow-brown, dry stroke. The other cultural characteristics agree with Sewerin's description. It may be well, however, to point out that there are many similarities in the growth of this organism with *Bact. Hartlebii.*

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* These Proceedings, 1899, Part 4, p. 661.

† Sewerin, Centralblatt für Bakt. ii. Abt. i., 162; iii., 510.

The main points of difference are that *Bact. Hartlebii* grows well in nitrate and ordinary bouillon at 37°, gas production is more vigorous at 22°, and it is a short rod without the vagaries of form which occur in *Vibrio denitrificans*.

The swollen organisms as depicted by Sewerin are similar to those found in cultures of *Rhizobium leguminosarum*. As I have shown in my paper on the latter organism, the appearance is due to a swollen gelatinous capsule which gathers round the junctions of the organisms.

It is unfortunate that Sewerin named the organism *Vibrio*. He apparently mistook the bent double cells for simple cells, and was influenced by Zettnow, who published drawings of *Vibrio rugula*, some of which are similar to the γ- and γ-forms of Sewerin's organism. I have already pointed out that *Rhizobium leguminosarum* is a budding fungus, and there is every reason to believe that *Vibrio denitrificans* is precisely of the same order. As it simulates a bacterium, the name *Mycobacterium denitrificans* would be more appropriate, while the species name is still maintained. *Mycobacterium*, as a name for those organisms which in cultures may assume a more or less mycelial character, has been suggested by Lehmann and Neumann.* In this group of organisms are included the plague, glanders. diphtheria, tuberculosis, and nodule organisms, all of which have been shown to produce, under certain circumstances. branching or mycelial forms.† According to Migula's classification, the order *Mycobacteriaceæ* develops Y-shaped forms with true branchings. Neither *Rhizobium leguminosarum* nor *Vibrio (Mycobacterium) denitrificans* forms true branching of the organisms, unless we agree to call everything within a single capsule an organism, and this

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* Lehmann and Neumann, Diagnostik. Chester, Studies in Systematic Bacteriology; Eleventh Annual Report Delaware College Agricultural Experimental Station, 1898-99.

† Skschivan, (Plague) Centralblatt für Bakt., i. Abt., xxviii., 289. Marx, (Glanders) *ibid.* xxv., 274. Schulze, (Tuberculosis) Zeitsch. für Hygiene xxxi., 153.

would be unwise. The definition requires modification to enable it to include organisms such as these which develop branching capsules. That the branching is caused by the capsules, and not by the simple organisms, can be seen from the plates which accompany this paper as well as my paper on "The Nodule Organism of the Leguminosæ."

EXPLANATION OF PLATE.

Vibrio (Mycobacterium) denitrificans.

Film from a 48 hours' culture in peptone-glucose-gelatine ($\times 1000$; the enlarged marginal illustrations are diagrammatic).